LEADING ARTICLE

Is Crohn's disease due to defective immunity?

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The understanding of the pathophysiology of Crohn's disease is currently undergoing a reassessment. The concept of this disease as a primary T cell disorder is being questioned, with a new emphasis on the role of innate immunity in initiating early events and in perpetuating the inflammatory state. Crohn's disease has been proposed instead to result from impaired innate immunity, encompassing the mucosal barrier and cellular elements including neutrophils and macrophages. Recent advances in genetics, functional studies on innate immunity and therapeutic trials on patients with Crohn's disease have lent support to this evolving hypothesis.

> "It's not what we don't know that hurts us, it's what we know for certain that just ain't so."
> Mark Twain

Until recently, the prevailing wisdom concerning the pathophysiology of Crohn's disease contended that the characteristic intestinal inflammation resulted from a T cell-driven process initiated by usually innocuous commensal bacteria or bacterial products. Crohn's disease has been viewed as a paradigm of a type 1 helper T cell, with raised levels of interferon γ and interleukin (IL)2.[1] The mucosal inflammation is theorised to be perpetuated by a failure to down regulate this immunologically activated state with a loss of normal tolerance to commensal flora. This conception has served as the guiding principle for most current and investigational agents that aim to suppress what is considered to be an inappropriate immune response, with most approaches targeting the T cell or T cell products.

However, several recent studies have prompted a major revision of this understanding of Crohn's disease, shifting the focus away from the T cell and adaptive immunity. The revised theory proposes that an aberrant innate immune response occurs more proximally, leading to T cell activation. The innate immune system is composed of inborn antimicrobial defences, not those dependent on prior exposure to a specific antigen as is required in the adaptive or acquired immunity. Both systems are linked and have overlapping pathways. However, innate immunity comprises a set of distinct elements, which includes circulating cells such as neutrophils, monocytes and resident intestinal immune cells (dendritic cells and Paneth cells) and also, importantly, intestinal epithelium and cellular products, including antimicrobial peptides such as defensins and cathelicidins. Different components of innate immunity in Crohn's disease have been suggested to be defective or impaired, a seemingly paradoxical hypothesis, given the data documenting Crohn's disease as an excessive inflammatory process often successfully treated with immune suppressants. However, several lines of evidence have converged to present a coherent hypothesis that impaired innate immunity initiates the cascade of events resulting in Crohn's disease. These advances include the genetics of Crohn's disease, with the association of nucleotide-binding oligomerisation domain (NOD)2/CARD15 in particular as a risk factor for Crohn's disease; recent insights into the functional implications of these genetic defects; investigations on the innate immune response in Crohn's disease; observations of Crohn's disease-like manifestations of genetic diseases of the innate immune system; and positive results of clinical trials of treatments that may stimulate innate immunity in patients with Crohn's disease. Although this hypothesis still rests on incomplete evidence, these data provide considerable support for the theory that Crohn's disease may result from an innate immune deficiency.

Although the neutrophil was investigated as possibly central to the pathophysiology of Crohn's disease decades ago, the ascendancy of the T cell eclipsed this early research. Recent genetic advances have again brought innate immunity to the forefront. The genetic association of NOD2/CARD15[2] with Crohn's disease established a critical link between the innate immune system, including circulating innate immune cells and the intestinal epithelium, and the development of the disease, but the nature of that pathway has remained controversial. On the basis of data from a rodent model, defective NOD2/CARD15 was suggested to lead to T cell activation through aberrant, dysregulated cytokine production due to loss of down regulation of toll-like receptor (TLR)2 signalling.[3] This account has been challenged. Rather than being a gain-of-function mutation, this mutation more probably imparts a loss of a variety of functions. The functional importance of NOD2/CARD15 seems to be in its binding to muramyl dipeptide (MDP), an important constituent of most bacterial cell walls, to sense and clear intracellular bacteria.[4] A defect in NOD2 leads to an attenuated induction of nuclear factor κB-dependent proinflammatory cytokine expression in different inflammatory cells isolated from patients. Monocytes homozygotic for NOD2/CARD15 (−/−) produced less IL8, IL1β and

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Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; MDP, muramyl dipeptide; NOD, nucleotide-binding oligomerisation domain; TLR, toll-like receptor

tumour necrosis factor α in response to MDP.[5] Dendritic cells homozygotic for NOD2/CARD15 (−/−), from patients with Crohn's disease, have a reduced expression of tumour necrosis factor α, IL12 and IL10 in response to MDP.[6] The most common allelic variant associated with Crohn's disease (3020insC) confers impairment of this bactericidal role in epithelial cells,[7] a different element of innate immunity. In addition, other critical proteins responsible for helping to protect the intestinal barrier, particularly defensins produced by Paneth cells, were shown to be decreased in patients with Crohn's disease and the deficiencies were proposed to be a central defect contributing to the development of Crohn's disease, independent of NOD2/CARD15.[8] NOD2/CARD15 defects, Paneth cell abnormalities and impaired defensin production have been proposed as an interaction that leads NOD2/CARD15 to be a risk factor most strongly for ileal Crohn's disease.[8–10]

In earlier years, innate immunity, particularly the neutrophil, was the focus of considerable attention as potentially the pivotal cell in the pathogenesis of Crohn's disease. Although the older literature was limited by methodological inadequacies, many of these studies detailed deficiencies of the innate immune function in patients with Crohn's disease, including impairments of neutrophil chemotaxis,[11] respiratory burst,[12] phagocytosis,[13] candidacidal capacity[14] and, perhaps most importantly, decreased bactericidal function.[15] Some of these studies have been recapitulated more recently with similar findings.[13 16]

In this context, Marks *et al*[17] have reported on a series of investigations that found a defective acute inflammatory response in the intestinal mucosa as well as in the skin of patients with Crohn's disease. The investigators assessed the acute inflammatory response provoked by a biopsy in normal-appearing ileal and rectal mucosa in healthy individuals and patients with Crohn's disease. After a repeat biopsy from the same site 6 h later, they found decreased accumulation of neutrophils in the patients with Crohn's disease compared with controls, with reduced levels of IL8, a cytokine critical for neutrophil recruitment, and also lower levels of IL1β. Although the relative IL8 deficiency and accumulation of neutrophils were independent of NOD2 genotype, when MDP was topically applied, cytokine levels increased in all patients with Crohn's disease except those who were homozygotic for the NOD2/CARD15 defect. Peripheral macrophages from patients with Crohn's disease with the NOD2/CARD15 mutation also showed impaired IL8 and IL1β secretion in response to various stimuli (lipopolysaccharides, tumour necrosis factor α, wound fluid and C5a), confirming similar earlier observations. As these models do not test the response to bacteria, heat-killed *Escherichia coli* were injected subcutaneously into the participants, and a depressed inflammatory reaction was seen in patients with Crohn's disease, attributed to diminished nitric oxide-dependent blood flow. The authors concluded that a systemic innate immune defect occurs in Crohn's disease, independent of NOD2/CARD15, which is associated with deficient acute neutrophil and macrophage responses and altered blood flow. This systemic defect does not account for the intestinal specificity of Crohn's disease or defects in epithelial function.

These studies are provocative and suggest new strategies for potential development of therapeutics aimed to normalise blood flow. Considerable evidence suggests that Crohn's disease may involve an endothelial defect with a resulting microthromboembolic disease.[18] Various factors, such as increased endothelin,[19] have been suggested to contribute to decreased blood flow. Prior studies had not linked the decreased blood flow to an inadequate inflammatory response. However, the investigators' conclusion that innate immune defects in Crohn's disease are centrally related to blood flow relies on an incomplete assessment of critical aspects of innate immune function. The central assay studied by Marks *et al*[17] was one of traumatic injury. Although followed up with studies examining blood flow in response to bacterial products injected subcutaneously, these assays do not necessarily adequately assess the capacity of the immune system to handle live bacteria that culminate in bactericidal function. The debate is of importance, as Marks *et al*'s findings suggest that the defective immune response could be potentially corrected by improving blood flow.

Marks *et al*[17] explored the possibility of sildenafil (Viagra, Pfizer, New York, New York, USA) as a potential drug for Crohn's disease on the premise that it would augment blood flow, increase accumulation of neutrophils and overcome the defect they have identified. Indeed, blood flow was improved by treatment with sildenafil in several patients with Crohn's disease. Although the result was not a clinical end point, sildenafil would be a surprising but welcome drug if it were broadly effective for Crohn's disease, given evidence of other functional deficits.

How the described impairments in innate immunity in patients with Crohn's disease may lead to the clinical disease remains inadequately understood. The broader functional consequences of such defects presumably would be to result in antigen or bacterial persistence, and consequently to T cell activation. However, the pathway leading to T cell activation and the cross talk between the innate immune system and the adaptive immune system in Crohn's disease are not well understood. The support for an innate immune insufficiency culminating in a Crohn's disease-like phenotype has been proposed on the basis of observations of the association of Crohn's disease or Crohn's disease-like intestinal manifestations with well-described genetic syndromes involving defects of innate immunity.[20–22]

Before the identification of the NOD2/CARD15 gene, Crohn's disease was proposed to result from genetic and environmental insults to the innate immune system, partly on the basis of examples from numerous diseases, such as chronic granulomatous disease, glycogen storage disease Ib and cyclic neutropenia.[20–22] Although most of these associations were case reports or series, a more systematic study found that 10 of 35 patients with glycogen storage disease Ib had radiological, endoscopic and histological features consistent with a Crohn's disease-like disease, including ileitis or colitis with skip lesions, stricturing disease, rectal sparing and perianal disease.[23] These reports support the idea that a variety of genetic defects in the innate immune system can result in a common Crohn's disease phenotype.[24] Mutations in NOD2/CARD15 may confer a milder but similar defect of innate immune function as those that occur in more severe genetic syndromes. Other genes suggested to be associated with Crohn's disease, such as TLR4,[25] OCTN1[26] and DLG5,[27] may also contribute to a defect in innate immunity through different mechanisms including changes in intestinal barrier function.[28]

The environmental influences contributing to innate immune impairment, in combination with a genetic predisposition, remain speculative.[24] Some have suggested that development of Crohn's disease has been influenced by a major shift in the intestinal flora of patients in developed countries in the 20th century. This change in intestinal microflora is evidenced by comparisons of gut flora from rural Africans and an urban English population.[29] The gut flora is increasingly dominated by *Bacteroides*, with diminished concentrations of bifidobacteria. Although controversial, this change in gut flora has been suggested to be more pronounced in patients with Crohn's disease.[30 31] Further, microbial differences in Crohn's disease may be related, partly, to deficiencies in antimicrobial peptide secretion.[8] Although these particular bacteria may be marker

species reflecting a broader change, these species may be important in themselves. Bifidobacteria[32] seem to augment innate immunity and *Bacteroides* contribute to its impairment.[33 34] Other suggested microbial changes in Crohn's disease, such as enteroadherent *E coli*, have been suggested to also disrupt the intestinal epithelial.[35] The so-called normal gut flora may have shifted from being essentially an accessory to the innate immune system to one that affects negatively the innate immunity, contributing to dysfunction. For individuals with certain genetic predispositions, this shift in flora may act synergistically to provoke Crohn's disease.

Other risk factors for the development of Crohn's disease, such as tobacco and non-steroidal anti-inflammatory drugs, have been shown to contribute to impairments to the innate immune system; tobacco reduces superoxide production[36] and non-steroidal anti-inflammatory drugs inhibit neutrophil chemotaxis[37] and superoxide production among other functions.[38] These environmental influences may only provoke Crohn's disease in those with a specific genetic risk. Consequently, a variety of environmental and genetic factors may target aspects of the innate immune system, and eventuate in a common end-stage process consistent with Crohn's disease.

These concepts of innate immune dysfunction formed the theoretical underpinnings for trials of granulocyte macrophage colony-stimulating factor[39] (GM-CSF, sargramostim; Leukine, Schering AG, Berlin, Germany) and granulocyte colony-stimulating factor (filgrastim; Neupogen, Amgen, Thousand Oaks, California, USA)[40] as drugs to treat Crohn's disease. Rather than acting to suppress T cell function, these compounds potentially augment the proximal, primary defect of innate immunity. Both agents were suggested to be beneficial when they were initially tested in open-labelled studies. Although it is difficult to compare two open-labelled studies, sargramostim seemed to be more effective and was selected for further studies. GM-CSF, a more potent stimulator of innate immune function than granulocyte colony-stimulating factor, is produced by the Paneth cells[41] and binds to intestinal epithelia,[42] suggesting its direct role in maintaining intestinal barrier function and intestinal innate immune function. A recent randomised controlled trial of sargramostim[43] in 124 people showed that individuals receiving sargramostim were more likely to have a beneficial response (defined by a decrease of 100 points in the Crohn's disease activity index) than those receiving placebo (48% *v* 26%) and remission (as defined by a Crohn's disease activity index of less than 150 points) (40% *v* 19%). Mechanistic studies were not part of the trial; however, a recent study[44] found that neutrophils from patients with Crohn's disease were deficient in respiratory burst and other assays. The addition of GM-CSF to neutrophils in vitro restored normal function. Other proposed treatments, such as microbial DNA products or probiotics more generally, may also stimulate innate immunity, perhaps partly through TLR9, although this has not been yet shown to have clinical benefit.[45]

In summary, increasing evidence supports the contention that Crohn's disease may result from an innate immune deficiency. Appreciation of the dysfunction contributed by NOD2/CARD15 mutations associated with Crohn's disease has provided insight into the pathophysiology of Crohn's disease as being possibly initiated by impaired innate immune function. Studies on molecular, cellular and animal models as well as in vivo investigations on patients with Crohn's disease have generated consistent data supporting this hypothesis. Numerous questions remain to be answered. Further functional studies on innate immunity continue to be critical. The environmental influences on innate immune function in patients with Crohn's disease are poorly defined. However, this evolving understanding of Crohn's disease may soon lead to clearer knowledge of the fundamental aetiology of this disease and thus to improved rational treatment therapies as well.

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